<Molecular Biofunction> Chemistry of Molecular Biocatalysts

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Scope of Research

Our research covers the comprehensive understanding of the physiological roles of biocatalysts (enzymes) as well as the reaction mechanism and specificity of each enzyme. **1**) Chemical, biochemical and molecular biological studies on diglycosidases specifically hydrolyzing the β-glycosidic bond between disaccharides and aglycons. **2**) Design and synthesis of transition-state analogue and mechanism-based inhibitors of γ-glutamyltranspeptidase and γ-glutamylcysteine synthetase. **3**) Design and synthesis of novel inhibitors of glycosidases and their application to affinity chromatography and biological probes to understand the physiological roles of glycosidases. **4**) X-ray crystallographic analysis of pyruvate phosphate dikinase from maize. **5**) Directed evolutional studies of *Pseudomonas* lipase. **6**) Mechanism of the activation/inactivation process of plant hormones by cytochromes P450. **7**) Molecular mechanism of regulation of phenylpropanoid pathway in plants subjected to various stresses.

Research Activities (Year 2003)

Presentations

Presentations from each project (**1** - **7**) are as follows:

1) Glycosidases in tea leaves, which may improve the flavor quality of CTC black tea, Sakata K, 3rd International Conference on Global Advances in Tea Science, Kolkata (India), 20-23 November.

2) γ-Phosphono glutamate analogs as mechanism-based inhibitors of γ-glutamyltranspeptidase, Hiratake J, Tachi N, Suzuki H, Kumagai H, Sakata K, 9th International Kyoto Conference on New Aspects of Organic Chemistry, 13 November.

3) Synthesis of cyclic glycosylamidine derivaives as β-*N*-acetylglucosaminidase inhibitor, Uno T, Kato M, Hiratake J, Sakata K, 2003 Annual and 431st Meeting of Kansai Branch of Jpn. Soc. Biosci. Biotech., and Agrochem., 5 October.

4) X-Ray crystallographic study on PPDK from maize, Nakanishi T, Nakatsu T, Matsuoka M (Nagoya Univ.), Sakata K, Kato H, 2003 Annual Meeting Jpn. Soc. Biosci, Biotech., and Agrochem., 1-3 April.

5) Directed evolution of lipase -Altering the reaction specificity and screening, Hiratake J, 3rd Combinatorial Bioengineering Symposium, 24 January.

6) Characterization of cytochromes P450 involved in ABA catabolism, Saito S, Matsumoto C, Hirai N, Ohigashi H, Ohta D, Mizutani M, Sakata K, 38th Annual Meeting Jpn. Soc. Chem. Reg. Plants, 29-30 October.

7) Metabolic profiling of coumarins in morning glory, *Ipomoea tricolor*, after various stresses, Kai K, Shimizu B, Sakata K, 30th Annual Meeting of Plant Growth Regulation Society of America, 3-6 August.

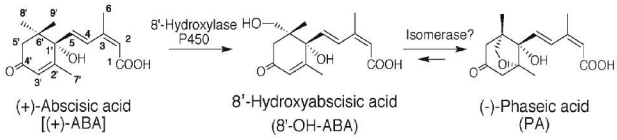
Grants

Sakata K, Clarification of a new group of plant diglycosidase family, Grant-in-Aid for Scientific Research (B) (2), 1 April 2001 - 31 March 2004.

Sakata K, Investigation of the floral aroma formation elicited by leaf-hopper feeding in Formosa Oolong Tea,

ABA catabolism by P450s

Abscisic acid (ABA) is a sesquiterpene plant hormone and is involved in a number of critical processes in normal growth and development as well as in adaptive responses to environmental stresses. The hydroxylation at the 8'-position of ABA has been known as the key step of ABA catabolism, and this reaction is catalyzed by ABA 8'-hydroxylase, a cytochrome P450 (P450) (Scheme 1). We have demonstrated *CYP707A*s as the P450s responsible for the 8'-hydroxylation of (+)-ABA. First, CYP707A cDNAs were cloned from *Arabidopsis* and used for the production of recombinant proteins in insect cells using a baculovirus system. The insect cells expressing CYP707A3 efficiently metabolized (+)-ABA to yield phaseic acid (PA), the isomerized form of 8'-hydroxy-ABA. The micorsomes from the insect cells exhibited very strong activity of 8'-hydroxylation of (+)-ABA (K_m = 1.3 μM and k_{cat} = 15 min_{-1}).

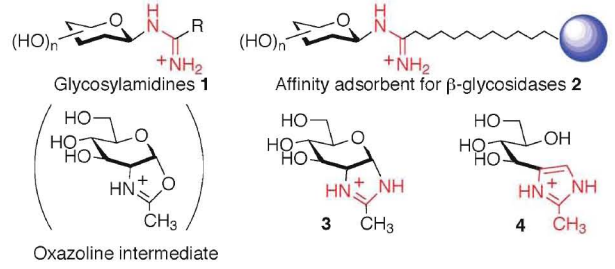


Scheme 1. The oxidative catabolic pathway of ABA

The solubilized CYP707A3 protein bound (+)-ABA with the binding constant K_s = 3.5 μM, but did not bind (-)-ABA. Detailed analyses of the reaction products confirmed that CYP707A3 does not have the isomerization activity of 8'-hydroxy-ABA to PA. The transcripts of CYP707A genes increased in response to salt, osmotic and dehydration stresses as well as ABA. These results establish that CYP707As play a key role in regulating the ABA level through the 8'-hydroxylation of (+)-ABA.

Design and synthesis of novel inhibitors of glycosidases

Glycosidases, one of the largest enzyme family, are composed of a number of enzymes with different substrate specificity and reaction mechanisms. Therefore the design of selective glycosidase inhibitors requires careful considerations for both substrate specificity and reaction mechanism of the glycosidase. We have developed β-glycosylamidines **1** as highly potent and selective inhibitors of β-glycosidases. The β-glycosylamidines, readily synthesized from sugars in two steps, serve as a highly inhibitory substrate analog (K_i < 0.1 μM) which inhibits each β-glycosidase selectively according to the glycon substrate specificity of the enzyme. This property was used successfully for the preparation of tailor-made affinity adsorbent **2** for the purification of glycosidases according to their glycon substrate specificity. We recently synthesized the cyclic glucosylamidine **3** which served as a highly potent and selective inhibitor of family 20 β-*N*-acetylglucosaminidases (IC_{50} = 0.17 μM). This



was because the compound **3** was positively charged and structurally analogous to the oxazoline intermediate, a cyclic reaction intermediate unique to this family of glycosidases. Compound **3** did not inhibit the conventional β-glycosidases which are inhibited potently by the generic glycosylamidine **1**. Interestingly, the acyclic imidazolo-sugar **4**, which was readily formed by isomerization of **3**, served also as highly potent and selective inhibitor of family 20 β-*N*-acetylglucosaminidases (K_i = 0.5 μM). The mechanism of inhibition, the structural elaboration and applications of these glycosidase inhibitors are studied actively in this laboratory.

Grant-in-Aid for Scientific Research (B) (2), 1 April 2003 - 31 March 2005.

Hiratake J, Bio- and organic chemical studies on glycosidases by using transition-state and substrate analogue inhibitors as a tool, Grant-in-Aid for Scientific Research (B) (2), 1 April 2001 - 31 March 2004.

Hiratake J, All-inclusive analysis of plant glycosidases by using specific inhibitors as a tool, Grant-in-Aid for Exploratory Research, 1 April 2003 - 31 March 2004.

Mizutani M, Analysis of the gene clusters involved in plant terpenoid biosynthesis, Grant-in-Aid for Young Scientist B, 1 April 2003 - 31 March 2005.

Awards

Kato M, Poster Award, Glycosylamidines as Potent and Selective Glycosidase Inhibitors, 1st International Symposium on Biomolecular Chemistry (ISBC2003), 5 December.

Saito S, Poster Award, Characterization of Cytochromes P450 Involved in ABA Catabolism, 38th Annual Meeting of Jpn. Soc. Chem. Reg. Plants, 30 October.